SEQUESTRATION OF CARBON DIOXIDE (CO_2) USING BIO-FOAMED CONCRETE BRICK INCORPORATING *BACILLUS TEQUILENSIS* BACTERIA

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ABSTRACT

The emission of carbon dioxide (CO_2) into the atmosphere increased rapidly in the last decades, which lead to global warming, climate change and rise in sea level. This research aimed to contribute on sequestration of CO_2 using biological approach to sequestrate CO_2 in bio-foamed concrete bricks (B-FCB). The used of biological approach in B-FCB is considered as new direction of CO_2 sequestration which in role helps to reduce the amount of CO_2 from atmospheric at the same time improve the strength and physical properties of B-FCB via precipitation of calcium carbonate ($CaCO_3$). The *Bacillus tequilensis* successfully isolated from cement kiln dust (CKD) to acclimatize in B-FCB. The *B.tequilensis* have the ability to produce carbonic anhydrase (CA) and urease enzymes to sequestrate CO_2 and convert it to $CaCO_3$. 2^k factorial and Response Surface Method (RSM) analysis were used to optimise carbonation depth and compressive strength of B-FCB using four factors, density (D), *B.tequilensis* (B), temperature (T) and CO_2. By given priority to carbonation depth the optimal carbonation depth and compressive strength of B-FCB used were 9.3 mm and 3.3 MPa at the following conditions; 20 % of CO_2, $3x10^5$ of B, 40 ºC of T and 1550 kg/m^3 of D at 28 days. The use of B in B-FCB increase CO_2 uptake by 29.7 % compared to foamed concrete brick without *B.tequilensis* (FCB) at 28 days. In addition, the use of B in B-FCB improve compressive strength, reduce initial rate of water absorption, water absorption and increase carbonation depth via 35.5 %, 76.4 %, 20.2 % and 30.0 % compared to FCB respectively. The change in chemical elements levels and increasing crystallinity precipitation in the form of $CaCO_3$ were analysed by SEM, EDX and XRD. Microstructure analysis confirmed the improvement of the compressive strength and physical properties of B-FCB compared to FCB, which was analysed via SEM, EDX and XRD.

ABSTRAK

Pencemaran karbon dioksida (CO_2) meningkat dengan cepat di atmosfera sejak akhir-akhir ini yang menyebabkan pemanasan global, perubahan iklan dan peningkatan paras air laut. Oleh itu, kajian ini berfokus kepada cara mengurangkan dan menstabilkan CO_2 dengan tujuan untuk mengatasi kesan negatifnya kepada manusia dan persekitaran.Pengasingan CO_2 boleh dilakukan dengan mengunakan kaedah geological. Namun, penstabilan geological adalah sangat mahal dan mungkin memberikan kesan kepada permukaan bawah tanah dan peningkatan pemendakkan kalsite pada air laut. Masa kini, saintis bertukar kaedah daripada menggunakan kaedah biologikal kepada kaedah baru untuk pengasingan CO_2. Tujuan kajian ini adalah untuk menggunakan kaedah biologikal untuk bata konkrit bio-berbusa (B-FCB). *Bacillus tequilensis* telah berjaya di asingkan daripada debu kilen simen (CKD) bagi disesuaikan dalam B-FCB. *B.tenquilensis* mempunyai kelebihan untuk menghasilkan anhydrase karbonatik (CA) and enzim urea bagi pengasingan CO_2 dan menukarnya kepada $CaCO_3$. Analysis faktor 2^k dan kaedah analisis permukaan tindak balas (RSM) digunakan untuk mengoptimumkan kedalaman karbonasi dan kekuatan mampatan bagi B-FCB menggunakan empat faktor iaitu density (D), B.tequnlesis (B), temperature (T), dan CO_2. Dengan memberi keutamaan kepada kedalaman karbonasi dengan maksima kedalaman karbonasi dan kekuatan mampatan bagi B-FCB dengan menggunakan 9.3 mm dan 3.3 MPa pada keadaan 20 % CO_2, $3x10^5$ *B.tequnlesis*, 40 °Cdan 1550 kg/m^3 untuk 28 hari. Penggunaan *B.tequnlesis* telah meningkatkan CO_2 sehingga 29.7 % dibandingkan dengan bata konkrit berbuih (FCB). Disamping itu, penggunaan *B.tequnlesis* pada B-FCB meningkatkan kekuatan mampatan mengurangkan kadar permulaan resapan air,mengurangkan kadar resapan air dan meningkatkan kedalaman karbonasi melalui 35.5 %, 76.4 %, 20.2 % dan 30.0 % dibandingkan dengan FCB. Perubahan level elemen kimia dan peningkatan pemendakkan kristial dalam bentuk karbonat kalsium ($CaCO_3$) telah di kesan yang di analisis menggunakan SEM, EDX dan XRD. Analysis struktur mikro telah mengesahkan terdapat penambahbaikan pada kekuatan mampatan dan ciri-ciri fizikal untuk B-FCB dibandingkan dengan FCB yang dianalysis menggunakan SEM, EDX dan XRD.

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INTRODUCTION

1.1 Background of study

Carbon dioxide (CO_2) concentration has increased in the current century compared to previous times due to increased anthropogenic activities and emissions sources [1]. The top four sources of atmospheric CO_2 emissions include; energy, transportation, industry and residential which emitted CO_2 by 41 %, 22 %, 20 % and 7 % respectively [2]. The current concentration of CO_2 has exceeded 400 ppm worldwide and is predicted to reach 650 ppm at the end of the century. This scenario is due to increasing anthropogenic activities and fewer developments of CO_2 sequestrations system [3]. Several technologies, including geological sequestration, chemical absorption, physical separation, membrane separation and biological fixation have been suggested to sequester atmospheric CO_2 [4][5]. Geological sequestration is the leading technology and represents the most useful approach to store large amounts of CO_2 underground or deep in the ocean [6][7]. However, the geological sequestration process is costly and slow in comparison to the amount of CO_2 released daily [8]. Therefore, the application of natural sequestration methods is urgently needed to reduce CO_2 and air pollution with consideration of the economic aspects.

Nowadays, the biological sequestration technique has attracted many types of research. These methods aim to evaluate the efficiency in removing CO_2 because the biological process is cheap and eco-friendly compared to geological sequestration technique [9]. Bio-sequestration is conducted by bacteria which has carbon anhydrase (CA) and urease enzymes, which act to accelerate CO_2 conversion to calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) through bio-mineralization [10]. This process occurs through the actions of bacteria in the soil that sequester CO_2. However, the chemical properties of the soil may negatively affect bio-sequestration [11].

Therefore, the process might be accelerated using the bio-augmentation process in which the bacteria is inoculated into the sequestration system.

Concrete play an important role on increase CO_2 concentration because concrete is the second most used material in the world after water. Whereas, the total generation of concrete raw materials ranges from 11.4 to 12.6 billion tons annually [12]. However, the increasing of concrete production confidently increases of cement content, which consider as one of the main sources of CO_2 production. That's because production of one ton of Portland cement clinker manufacture is responsible for releasing about one ton of CO_2 in atmospheric [13]. However, concrete could be used as CO_2 reservoir if carbonation process of concrete accelerates and can reduce the emitted CO_2 from cement factors if cement content is replaced via other pozzolanic materials such as; fly ash, coal ash, biomass ash, palm oil ash, rice husk ash and limestone powder est., to minimize the energy consumption and greenhouse gasses emission [14][15][16][17][18]. Therefore, minimization of CO_2 amount in atmospheric, can be done by decrease the emission of CO_2 that coming from cement factors and other industrials sources or by sequestration the amount of CO_2 that already available in the atmospheric via acceleration carbonation process in concrete.

Carbonation of concrete could be one of the natural ways that may contribute on sequestration process, which has the ability to sequestrate CO_2 by reaction of Portland cement in the presence of moisture in the form $CaCO_3$ [19]. However, carbonation process is very slow and depends on chemical and physical properties of solid also on exposure conditions of the concrete. In more details, concentration of CO_2, relative humidity, types and size of materials, pH value, concrete porosity, water cement ratio, temperature and surface area all are factors affect carbonation process [20][21]. Therefore, the factors restricting carbonation process must be known if there is an intention to use carbonation as a natural way to sequestrate CO_2 in concrete. However, carbonation may affect the reinforcement and cause corrosion. When the pH value drop in concrete the passive protective oxide film around the steel exposed to destroy resulted steel corrosion [22]. The high depth of carbonation occurred with higher drop of pH value. Therefore, pH value can be used as prediction factor of carbonation process. Therefore, foamed concrete bricks (FCB) could be one of the appropriate applications to sequestrate high amount of CO_2 via acceleration of carbonation process because there is no steel in FCB and it has high level of porosity. However, natural carbonation is very slow as demonstrate in the previous paragraph.

Therefore, the used of bacteria in FCB to produce new type of concrete bricks namely bio-foamed concrete bricks (B-FCB) could be more useful to accelerate carbonation process, which can be considered as new application of biological sequestration. According to previous studies in bio-concrete technology, numerous bacterial species such as; *Bacillus pasteurii, Pseudomonas aeruginosa, B. alkalinitrilicus, B. sphaericus, B. subtilis, Enterococcus faecalis, Shewanella* sp.*, S. pasteurii* and *Ureolytic* bacteria have been applied to enhance bio-concrete properties via precipitation of $CaCO_3$ in the pores [23][24][25][26][27][28][29]. The selected bacterial strain should have the ability to produce carbonic anhydrase (CA) and urease enzymes in the concrete to accelerate the carbonation process and CO_2 sequestration [30].

The previous statements give a brief discussion about the effect CO_2 emission to the environment and the role of cement production on increasing CO_2 emissions. At the same time, the technology used to sequestrate CO_2 is not enough to sequestrate the available CO_2 in atmospheric. Therefore, the use of biological sequestration to accelerate carbonation process in B-FCB could be one of the mitigation techniques used to sequestrate CO_2 in the future. Therefore, the factors affecting carbonation process as well as the factors affecting growth activity in B-FCB must be in consideration. Furthermore, bacteria should have the ability to produce CA and urease enzyme, which have the ability to capture and immobilize CO_2 from atmospheric in the form of $CaCO_3$. This approach is to find the optimisation of CO_2 sequestration into B-FCB via carbonation process.

1.2 Problem Statement

The emission of CO_2 into the atmosphere increase worldwide. While, the sequestration techniques used such as planting, geological and biological sequestration are not sufficient to sequestrate the emitted CO_2. Geological direction has the ability to sequestrate high amount of CO_2, however, the process of this type takes long time and very expensive in comparison with biological sequestration [4][31][32][33]. Therefore, biological sequestration is recently used worldwide because it is considered as natural way to sequestrate CO_2 using microorganisms especially those that have the ability to produce CA or urease enzymes [34][35].

This finding suggest that bacteria has high potential to use bio-concrete as new application of biological sequestration. Because, bacteria has the ability to produce CA and urease enzymes, which in role help on accelerate carbonation process and sequestrate CO_2 from the atmosphere and precipitate it in the form of $CaCO_3$ in concrete pores [36]. However, most of the researchers consider carbonation as a serious problem for the concrete because it decreases the acidity in concrete to about 9 pH, which destroys the passive oxide film leaving the steel exposed to corrosion [1][37]. Therefore, most of the researchers in bio-concrete technology realized the great potential of bacteria in bio-concrete such as increment of compressive strength through self-healing process of porosity. Nonetheless, they did not report the ability of bacteria to accelerate carbonation process because the increase of carbonation may lead to corrosion of concrete bars [38]. Thus, this research applied the use of bacteria that has the ability to produce CA and urease enzymes to accelerate carbonation process and enhance the properties of B-FCB.

On the other hand, the extreme pH and anaerobic conditions are considered as unsuitable environment for bacteria within the concrete. Due to this challenge, microcapsules were used as bacterial carriers in the concrete to resist high pH of concrete and sensitive humidity [36][39][40]. Other researchers attempts to solve the problem by protecting the bacteria from strong alkaline condition in concrete by immobilizing the bacteria in silica gel [41]. Bacteria can also be isolated in high alkaline and anaerobic conditions to acclimatize in concrete environment which is added to bio-concrete mixture in the form of liquid (*liquid culture*) [42]. However, the used of liquid culture which consist nutrient growth may be reactive to other bacteria in concrete materials. Accordingly, bacteria should have the ability to survive in B-FCB conditions to be able to accelerate carbonation process. In addition, there are many factors affecting carbonation of concrete, which are divided into three categories as follow; physical properties of the solid, chemical properties of the materials and external conditions of the environment [43]. The density of concrete and curing conditions such as temperature and CO_2 concentration are the most factors affection the carbonation in concrete [21][44]. Therefore, the effect of bacteria and the other factors affecting carbonation of B-FCB must be studied and consider as main factors to optimize sequestration of CO_2 using B-FCB.

The statements above illustrates that, the use of bacterial species in B-FCB systems has a potential biological sequestrate of CO_2 and can be considered as a future strategy to reduce the high CO_2 pollution. Bacterial cells can capture CO_2 by accelerating the carbonation processes, which convert CO_2 to calcium carbonate ($CaCO_3$) via carbon anhydrase enzyme and indirect role for urease. Moreover, the stress environmental conditions in the B-FCB require more attention to find selected bacterial strain, which has high potential to survive and grow in these conditions.

1.3 Objectives of research

This research is aimed at studying the potential of B-FCB to sequestrate CO_2 from the atmosphere into concrete pores by carbonation process. The study sets out to achieve its aim based on the following objectives:

1. To identify the most potent bacteria strain from cement kiln dust (CKD) for CO_2 sequestration in B-FCB.
2. To optimize the compressive strength and CO_2 sequestration by carbonation depth in B-FCB.
3. To propose the empirical models for optimum CO_2 sequestration and compressive strength of B-FCB.
4. To evaluate the compressive strength and physical properties, and morphology of microstructure of FCB and B-FCB after the CO_2 sequestration process.

1.4 Scope of study

This research is based on the experimental work on the use of bacteria in bio-foamed concrete in order to sequestrate CO_2 and improve properties of B-FCB. Laboratory activities conducted in environmental and material labs with six phases of study.

Phase 1: The activities involved collection of bacteria samples. The samples were collected from cement kiln dust, which located in Cement Industries of Malaysia Berhad (CIMA) to be able to adopt on foamed concrete environment and high concentration of CO_2.

Phase 2: This phase aimed to isolate the bacteria from the collected samples in environmental lab to acclimatize on B-FCB environment. The medium selected for isolate bacteria colonies and purified according to Al-Gheethi [45], whereas, the incubation process was conducted at 30 °C. Gram statin method used to confirm the purification of bacterial cells.

Phase 3: This phase focused on selecting the most potent bacteria. Whereas, several assays conducted to select the most potent bacteria such as; enzymatic assays to confirm the ability of bacteria to produce CA and urease enzymes, measure the abundant of bacteria growth, observed the growth of bacteria with high level of CO_2 and growth of isolated bacteria in simulation medium of B-FC. The assay of CA enzyme among the bacterial isolates was carried out using tubes technique on culture Trypticase Soy Agar medium (TSA). On the other hand, the potential of isolate bacteria to produce urease enzyme tested following the description by Benson [46]. The last step in this phase is preparation and autoclaved synthetic medium containing the foamed concrete materials as follow; water, sand, cement and foamed agent to simulate the B-FCB environment and make sure of bacteria growth. Finally, produce bacteria in powder form using dry freezing method was produced.

Phase 4: This phase concentrated to study plastic stage of foamed concrete with and without bacteria (workability). Therefore, workability measured using fresh density test and inverted slump test according to ASTM C995, 2001. After that, optimisation of compressive strength and carbonation depth were analyzed. Screaming stage started with 11 runs as suggested by 2^k factorial design using Minitab 18 software to study the significant effect of the following factors; CO_2 concentration, *B.tequilensis* concentration, temperature (T) and B-FCB density (D) to compressive strength and carbonation depth of B-FCB. RSM method also suggested more 10 runs to optimize compressive strength and carbonation depth of B-FCB. Therefore, the total runs used in this research was 21 runs.

Phase 5: This phase focused on propose empirical model equation for CO_2 sequestration by carbonation depth and the compressive strength of B-FCB, which improved using RSM methods in Minitab 18.

Phase 6: This phase aimed to study hardening stage of FCB and B-FCB specimens with size (215 mm x 100 mm x 65 mm) which prepared for compressive strength, initial water absorption, water absorption and carbonation depth according to the following standards; BS12390 – 6:2000, BS 12390 – 3:2002, BS 12390 – 8:2000 respectively [41-43]. The curing process of tests specimens conducted on dry condition in side chamber with different concentration of CO_2 and temperature ˚C according to the suggested conditions via 2^k factorial and RSM method.

Phase 7: This phase carried out the microstructure analysis of the samples of FCB and B-FCB. Microstructure analysis conducted in this study by the following tests; energy dispersive X-ray (EDX) to evaluate the change in chemical elements, X-ray diffraction analysis (XRD) to evaluate the percentage of crystallinity and scanning electronic microscopy (SEM) to investigate healing process of the pores [47][5][48].

1.5 Significance of study

Previous studies shown that, there is no much or non-study on CO_2 sequestration into bio-concrete. Therefore, the novel idea in this research is the used of *B.tequilensis,* which has the ability to produce CA and urease enzymes and apply in B-FCB as one option of natural CO_2 sequestration. The selection of CKD as *B.tequilensis* source samples were due to the following reasons; the environment in cement factory is very rich of CO_2 and the samples of CKD almost contains the same chemical composition of cement. These two reasons help on produce bacteria capable to adopt in B-FCB environment and has the ability to accelerate sequestration of CO_2. On the other hand, some of the previous studies used bacteria in the form of liquid media which consist of nutrient broth, whereas, the availability of nutrient broth in bio-concrete mixture may re-active other bacteria that naturally available in concrete materials to grow, which may affect the properties of bio-concrete. Therefore, the used of *B.tequilensis* in powder form was to avoid the growth of other bacteria and help on controlled the concentration of *B.tequilensis* cell in concrete mixture. Storage of CO_2 in B-FCB via *B.tequilensis* may help to increase the precipitate of $CaCO_3$, which in role help to improve B-FCB properties via healing process. Therefore, this research aimed to contribute on reducing of CO_2 from atmospheric at the same time enhance B-FCB properties.

1.6 Organization of thesis

This thesis mainly consists of six chapters which are illustrated as follow:

Chapter one, presents the background of study, problem statement, objectives of study, scope of work and significance of study.

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